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SJSU Undergraduate Research Grants

Understanding the Allosteric Regulation of SIRT1 on Different Substrates

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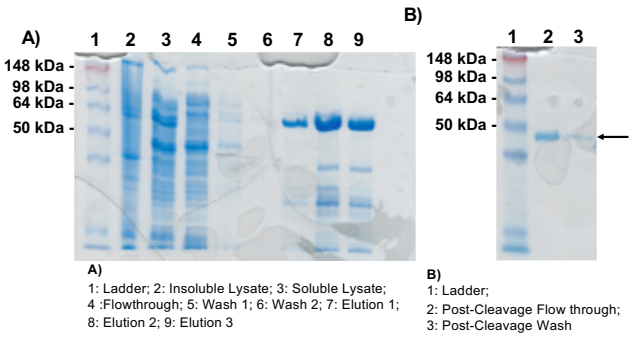
Abstract

SIRT1 is an NAD^+-dependent protein deacetylase that plays an important role in pathological and physiological events.[1] The purpose of this lab is to study how the unstructured N-terminus domain of SIRT1 regulates its catalytic activity in a substrate-specific manner. To have a better understanding, we will observe how resveratrol, a small molecule, modulates SIRT1 activity. Resveratrol is a natural phenol product from plants that has been shown to modulate SIRT1 activity by interacting with N-terminus domain of SIRT1. Recent studies have shown that resveratrol has different effects on SIRT1 activity based on the different acetylated peptide substrates used, including increase, decrease, and no change in deacetylation activity. We hypothesize that the conformational change of the N-terminal domain affects the ability of SIRT1 to recognize its substrates. To test this hypothesis, peptides are selected based on three categories where SIRT1 deacetylation activity was activated, deactivated, or unchanged by resveratrol. SIRT1 activity towards these peptides will then be examined with coupling assay to obtain their k_{cat} and K_M values. These values can determine the role that the N-terminus plays in SIRT1 deacetylation activity. Our results reveal how resveratrol contributes in affecting the N-terminus domain of SIRT1 to modulate its enzyme activity. Collectively, the understanding of substrate-specific regulation of SIRT1 by resveratrol may serve as further information for designing target-specific drugs for therapeutic purposes.

Project Activities or Findings

- SIRT1 was purified by Ni-NTA affinity purification.
- Resveratrol's effects on SIRT1 activity were determined by enzyme activity assays and the Michaelis-Menten kinetic parameters were determined:
  A change in k_{cat} would suggest alterations in the catalytic rate, whereas changes in K_M would suggest that substrate recognition is affected.
- Assays on the peptide Ac-CSNK show a decrease of SIRT1 activity by ~3 fold after addition of resveratrol.
  The significant change in K_M could suggest that resveratrol affects the substrate recognition of SIRT1.



A)
1: Ladder; 2: Insoluble Lysate; 3: Soluble Lysate; 4 :Flowthrough; 5: Wash 1; 6: Wash 2; 7: Elution 1; 8: Elution 2; 9: Elution 3

B)
1: Ladder;
2: Post-Cleavage Flow through;
3: Post-Cleavage Wash

Figure 1. A) SDS-PAGE of first step of Ni-NTA purification. **B)** SDS-PAGE of second step of purification after cleavage of the SUMO solubility tag. SIRT1 in second lane marked by arrow (m.w.= 46 kDa).

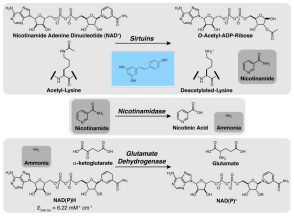


Figure 2. Overall scheme of the enzyme-coupled assay used to quantify SIRT1 activity.[4]

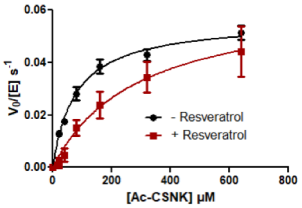


Figure 3. Enzyme kinetics graphs and parameters of SIRT1's activity on Ac-CSNK with and without resveratrol shows a significant change in K_M. Black curve (no res) shows as an average of triplicates. Red curve (+ res) shows as an average of quadruplicates. Resveratrol was added to a final concentration of 200 μM.

Substrate/Regulator Combo	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M ($\mu M^{-1}s^{-1}$)
Ac-CSNK	0.056 ± 0.002	77.50 ± 8.611	(7.201 ± 2.341) x 10^{-4}
Ac-CSNK + resveratrol	0.065 ± 0.014	289.1 ± 140.9	(2.239 ± 1.035) x 10^{-4}

Research Questions

- How does resveratrol affect SIRT1's activity with various peptide substrates?
- Is SIRT1's activity affected by changes in k_{cat} or K_M values?

Acknowledgments

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Citations

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